Inhibition of Acid-induced Lung Injury by Hyperosmolar Sucrose in Rats

Zeenat Safdar, Maimiti Yiming, Gabriele Grunig, and Jahar Bhattacharya

Lung Biology Laboratory, Division of Pulmonary-Critical Care Medicine, and Department of Pathology, College of Physicians and Surgeons, Columbia University, St. Luke's–Roosevelt Hospital Center, New York, New York

Rationale: **Acid aspiration causes acute lung injury (ALI). Recently, we showed that a brief intravascular infusion of hyperosmolar sucrose, given concurrently with airway acid instillation, effectively blocks the ensuing ALI.**

Objectives: **The objective of the present study was to determine the extent to which intravascular infusion of hyperosmolar sucrose might protect against acid-induced ALI when given either before or after acid instillation.**

Methods: **Our studies were conducted in anesthetized rats and in isolated, blood-perfused rat lungs. We instilled HCl through the airway, and we quantified lung injury in terms of the extravascular lung water (EVLW) content, filtration coefficient (K_{fc}), and cell counts and protein concentration in the bronchoalveolar lavage. We infused hyperosmolar sucrose via the femoral vein.**

Results: **In anesthetized rats, airway HCl instillation induced ALI as indicated by a 52% increase of EVLW and a threefold increase in K_{fc}. However, a 15-min intravenous infusion of hyperosmolar sucrose given up to 1 h before or 30 min after acid instillation markedly blunted the increases in EVLW, as well as the increases in cell count, and in protein concentration in the bronchoalveolar lavage. Hyperosmolar pretreatment also blocked the acid-induced increase of K_{fc}. Studies in isolated perfused lungs indicated that the protective effect of hyperosmolar sucrose was leukocyte independent.**

Conclusions: **We conclude that a brief period of vascular hyperosmolarity protects against acid-induced ALI when the infusion is administered shortly before, or shortly after, acid instillation in the airway. The potential applicability of hyperosmolar sucrose in therapy for ALI requires consideration.**

Keywords: acid aspiration syndrome; extravascular lung water; filtration coefficient; leukocyte count; lung blood content

The aspiration of gastric acid, which is prone to occur in conditions such as drug overdose, stroke, and general anesthesia, is an increasingly important cause of acute lung injury (ALI), which is associated with high rates of morbidity and mortality (1). In animal models of acid-induced ALI, intratracheal acid instillation induces lung leukocyte sequestration that characteristically precedes pulmonary edema (2, 3). Accordingly, proposed therapeutic strategies include protocols that either inhibit lung leukocyte accumulation (4–8) or reduce alveolar liquid accumulation (9–11). However, strategies for lung endothelial barrier strengthening remain inadequately considered.

(*Received in original form January 1, 2005; accepted in final form July 12, 2005*)

Supported by National Institutes of Health grants HL-36024, HL-57556, and HL-64896 (J.B.), and by grants from New York State's Empire Clinical Investigator Program and Stony Wold-Herbert Fund, Inc. (Z.S.).

Correspondence and requests for reprints should be addressed to Jahar Bhattacharya, M.D., D.Phil., St. Luke's–Roosevelt Hospital Center, 1000 10th Avenue, New York, NY 10019. E-mail: jb39@columbia.edu

Am J Respir Crit Care Med Vol 172. pp 1002–1007, 2005
Originally Published in Press as DOI: 10.1164/rccm.200501-005OC on August 18, 2005
Internet address: www.atsjournals.org

The lung endothelial barrier, which is established by intercellular junctional proteins of endothelial cells lining lung microvessels, protects against excessive lung liquid accumulation. Decreased lung endothelial barrier function increases transmicrovascular liquid flux, thereby promoting pulmonary edema, which is a hallmark of ALI. Although it has long been known that airway acid instillation injures the lung microvascular barrier (12), relevant therapeutic strategies are not known. This article considers the possibility that a brief period of plasma hyperosmolarity protects against acid-instilled lung microvascular injury.

This consideration is based on our recent report that a 15-min infusion of hyperosmolar sucrose, which increases vascular osmolarity by approximately 50 mOsm, strengthens the lung endothelial barrier, and enhances actin polymerization in the endothelial periphery (13, 14). Potentially, these are antiinflammatory effects, because the barrier strengthening blocks edema formation, whereas the actin enhancement inhibits leukocyte accumulation by blocking expression of the leukocyte adhesion receptor P-selectin (13). Importantly, these endothelial responses are maintained for up to 2 h after a single sucrose infusion.

We also showed in this study that the sucrose infusion protected against acid-induced ALI, provided we gave the infusion concurrently with airway acid instillation (13). Hence, our present aim was to determine the extent to which hyperosmolar sucrose might protect against the injury when given either before or after acid instillation. We report below our findings that, in both instances, hyperosmolar sucrose was protective against ALI.

METHODS

Solutions

All reagents were obtained from Sigma Aldrich (St. Louis, MO) unless otherwise specified. Isosmolar Ringer's buffer contained 4% albumin (essentially fatty-acid free) plus Na^+ 144 mM, Ca^{2+} 1.5 mM, Cl^- 123 mM, and lactate 28 mM. Hyperosmolar sucrose was prepared by adding sucrose to isosmolar Ringer's buffer (365 g/L) to achieve osmolarity of 1,500 to 1,600 mOsm/kg, as determined by freezing-point depression osmometry (Advanced Instruments, Norwood, MA).

Anesthetized Rat

We conducted all experiments on adult male Sprague-Dawley rats (500 g) in accordance with protocols approved by our institutional animal care and use committee. In each experiment, we anesthetized rats using a combination of halothane (4%) and sodium pentobarbital (intraperitoneal injection, 35 mg/kg) (13, 15). We maintained body temperature at 37°C by placing the animals on a warming blanket (Harvard Apparatus, Holliston, MA). We introduced catheters (PE 10; Becton Dickinson, Parsippany, NJ) in the femoral artery and femoral vein to record arterial blood pressure and heart rate (PowerLab; ADInstruments, Colorado Springs, CO), to obtain arterial blood samples for blood gas analyses (ABL 5; Radiometer America, Inc., Westlake, OH), and to give intravenous infusions. To instill acid or saline in the lung, we introduced a cannula via a tracheotomy.

In the test group, we gave a 15-min infusion (4 ml/kg) of hyperosmolar sucrose via the femoral vein catheter. At different times after the femoral infusion, we instilled 0.1N HCl (2 ml/kg) via the tracheal cannula. In the control group, we followed identical femoral vein infusion and tracheal instillation procedures, except we infused Ringer's buffer

of physiologic osmolarity and we instilled saline via the tracheal cannula. Two hours after airway instillation, we quantified the blood-free extravascular lung water content (EVLW).

Isolated Blood-perfused Lung

Using our previously reported methods (13, 16), we established the isolated blood-perfused rat lung preparation, which we maintained at constant blood flow (12 ml/min) at pulmonary artery and left atrial pressures of 10 and 3 cm H_2O, respectively. We continuously ventilated the lungs at inspiratory and expiratory pressures of 11 and 4 cm H_2O, respectively, and at a tidal volume of 6 ml/kg (17). To determine the role of leukocytes, we obtained leukocyte-free blood by centrifuging whole blood (800 g, 10 min, 4°C) and then removing the buffy coat from the supernatant. In blood aliquots, we determined counts of leukocytes fluorescently labeled with rhodamine 6 G (5 μg/ml, 10 min) in a hemocytometer under fluorescence microscopy.

Lung Filtration Coefficient

The methods described here have been established in our laboratory (18). We placed the isolated blood-perfused lung on a pan suspended from a force-displacement transducer (model FT 10; Grass Instrument Co., Quincy, MA). We calibrated the transducer using standard weights and set amplifier gain such that a 1-cm deflection on the tracing reflected a 200-mg change of lung weight. For lung filtration coefficient (K_{fc}) determination, we adjusted vascular pressures to establish isogravimetric conditions such that lung weight remained unchanged for at least 20 min. Then, we increased the left atrial pressure by 5 cm H_2O for 1 min to induce the well-reported fast and slow phases of weight gain (18). We quantified the rate of change of weight ($\Delta W/\Delta t$) in the slow phase and factored it by the pressure increase and the lung weight to express K_{fc} as ml/(min · cm H_2O · 100 g lung weight).

EVLW and Lung Blood Content

Except where stated, 2 h after intratracheal liquid instillation we determined the blood-free EVLW content by the method of Selinger and colleagues (19), which we have used several times (20, 21). Briefly, in this approach, the wet–dry ratio of the lung homogenate is corrected for the blood water content and EVLW is expressed as g/(g blood-free dry weight). Accordingly, aliquots of lung homogenate are subjected to centrifugation and the lung blood content (Qb) is estimated as (Qh × [Hb]s]/([Hb]h × Fwh/Fws), where Qh is the weight of the aliquot of homogenate, (Hb)s is the supernatant hemoglobin concentration, (Hb)h is the blood hemoglobin concentration, Fwh is the fractional water content of the homogenate, and Fwh is the fractional water content of the supernatant.

Bronchoalveolar Lavage

For bronchoalveolar lavage (BAL), we instilled via the tracheal cannula 15 ml of sterile, pyrogen-free Hanks buffer, in three 5-ml aliquots. For each lavage, we recovered 12.5 to 13 ml of BAL fluid. We pooled the BAL samples, and we counted the total number of cells in a hemocytometer. To obtain neutrophil counts, we centrifuged (Cytospin 2; Shandon Lipshaw, Inc., Pittsburg, PA) BAL samples, then stained them with Wright and Giemsa solutions. Differential counts were performed on 200 cells using standard morphologic criteria. To determine protein concentration, we centrifuged the lavaged fluid for 10 min at 4°C and determined protein concentration of the BAL supernatant by the bicinchoninic acid assay (Pierce, Rockford, IL).

Statistics

All data are reported as mean ± SD unless stated otherwise. Differences between groups were determined by using paired Student's *t* test, by analysis of variance (Newman-Keuls test), as well as by the nonparametric rank test. Significance was accepted at a p value of less than 0.05.

RESULTS

Anesthetized Rat

Osmolarity. At the end of a 15-min femoral vein infusion of hyperosmolar sucrose, plasma osmolarity increased 55 ± 13 mOsm/kg above the preinfusion level (Figure 1). Subsequently, osmolarity returned to baseline within 15 min. The infusion of hyperosmolar sucrose did not cause statistically significant changes in mean arterial pressure, heart rate, or respiratory rate (Table 1). Blood hemoglobin and hematocrit also remain unchanged, indicating that the sucrose infusion did not cause hemolysis. In two experiments, the total leukocyte count did not change.

EVLW. We intravenously infused hyperosmolar sucrose for 15 min. At different intervals after the end of the infusion, we instilled 0.1N HCl (2 ml/kg) into the trachea. In control experiments, we injected isosmolar buffer instead of sucrose, and we instilled saline instead of acid. In the isosmolar group, 2 h after acid instillation, Po_2 was markedly lower than the preacid control (Table 1). However, in the hyperosmolar group, no postacid decrease of Po_2 occurred (Table 1), indicating that hyperosmolar pretreatment protected blood oxygenation.

Similar to our previous data (13), in lungs removed 2 h after saline instillation, EVLW was higher than in untreated control lungs ($p < 0.05$), probably because of incomplete removal of the instilled liquid. However, in these saline-instilled lungs, EVLW was unaffected by hyperosmolar infusion (Figure 2) (13).

Acid instillation increased EVLW two times above that for saline instillation (Figure 2). The acid-induced EVLW increases were completely inhibited by hyperosmolar sucrose infusions given 0.5 or 1 h, but not 2 h, before acid instillation (Figure 2). Postinstillation EVLW was lower in the inhibited groups than in the saline-instilled group ($p < 0.05$; *see* DISCUSSION). Hyperosmolar urea infusion given 0.5 h before acid instillation did not block the acid-induced EVLW increases (Figure 2).

In separate experiments, we determined the effect of hyperosmolar sucrose given after establishing ALI. EVLW increased progressively after acid instillation, being 20% higher than saline-instilled controls 0.5 h after instillation, but more than 50% higher after 2 h (Figure 3), indicating progressive development of ALI. However, when we gave hyperosmolar sucrose 0.5 h after acid instillation, the increase of EVLW at 2 h was markedly blunted. In fact, this blunted EVLW was not different from EVLW obtained 0.5 h after acid instillation in the untreated group (Figure 3). We interpret that hyperosmolar infusion given after acid instillation prevented progressive ALI.

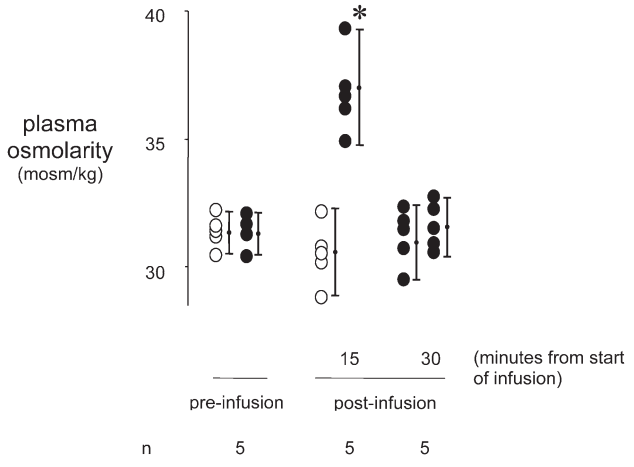

Figure 1. Plasma osmolarity after intravenous infusions. Osmolarity is shown for plasma samples obtained before and after, as indicated, 15-min intravenous infusions of isosmolar buffer (*open circles*) or hyperosmolar sucrose (*filled circles*). *Time points* denote period elapsed from the start of infusion. Mean ± SD. n, number of experiments. *$p < 0.05$ compared with preinfusion.

TABLE 1. EFFECT OF INTRAVENOUS HYPEROSMOLAR SUCROSE ON CARDIORESPIRATORY VARIABLES

Infusion	Acid Instillation	n	MBP (*mm Hg*)	HR (*beats/min*)	RR (*/min*)	Hb (*g/dl*)	Hct (%)	Arterial P_{O_2} (*mm Hg*)
Untreated		5	99 ± 3	312 ± 7	83 ± 2	15.9 ± 0.4	47 ± 1	94 ± 11
Isosmolar buffer	Before*	5	108 ± 2	310 ± 10	77 ± 3	14.9 ± 0.9	45 ± 2	94 ± 7
	After†	5	102 ± 2	318 ± 27	85 ± 5	14.6 ± 1	43 ± 3	49 ± 3§
Hyperosmolar Sucrose	Before*	6	101 ± 4	288 ± 18	79 ± 6	13.4 ± 0.2‡	47 ± 3	86 ± 9
	After†	6	102 ± 5	355 ± 21	81 ± 7	13.6 ± 0.1‡	48 ± 2	81 ± 4

Definition of abbreviations: Hb = hemoglobin; Hct = hematocrit; HR = heart rate; MBP = mean arterial blood pressure; n = the number of experiments; RR = respiratory rate.

Values are mean ± SD.

* Data obtained immediately before acid instillation.

† Data obtained 2 h after acid instillation.

‡ $p < 0.05$ as compared with untreated group.

§ $p < 0.05$ as compared with isosmolar preacid group.

Plasma hyperosmolarity induces lung erythrocyte retention (22), which could cause an overestimate of the lung's blood-free dry weight (23), leading to errors in the EVLW estimate. However, in the present experiments, hyperosmolar infusion did not cause lung erythrocyte accumulation, because it did not change lung blood content as compared with untreated, or isosmolar, controls (Figure 4, *first three groups* of data points from *left*). Although acid instillation increased lung blood content (Figure 4, *fourth group*), affirming a previous report that acid injury induces lung erythrocyte accumulation (24), hyperosmolar infusion given 30 min before acid markedly blunted the increase of lung blood content. Hence, hyperosmolar sucrose infusion given before acid protected against lung erythrocyte accumulation.

BAL. To determine the extent of lung leukocyte recruitment and protein permeability, we quantified total cell count, neutrophil count, and protein concentrations in BAL. At baseline, these variables were consistent with previously reported data (Figure 5) (7, 25, 26). We gave a 15-min infusion of isosmolar buffer, then we followed 0.5 h later with intratracheal acid instillation. BAL obtained 2 h after acid instillation showed increases in all variables. However, the BAL was not blood stained, indicating that acid instillation did not cause frank hemorrhage into the alveolar spaces. A 15-min infusion of hyperosmolar sucrose given either 0.5 h before or 0.5 h after acid instillation completely blocked the increases of BAL protein and cells (Figure 5). These findings indicate that vascular hyperosmolarity prevented acid-induced increases in lung leukocyte recruitment and alveolocapillary permeability to plasma proteins.

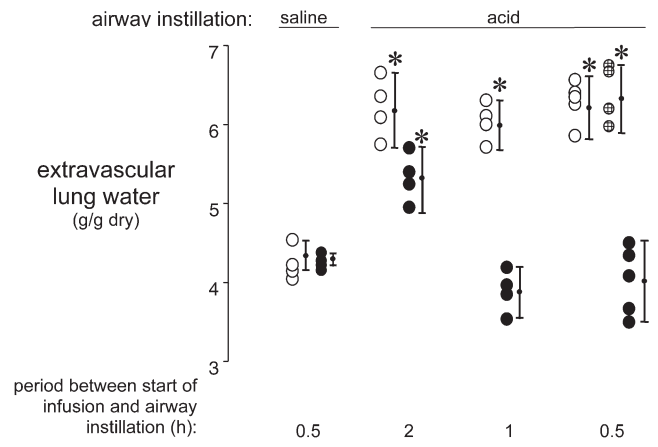

Figure 2. Preacid infusion of hyperosmolar sucrose. Levels of extravascular lung water are shown for groups given either airway instillation of saline or acid as indicated. Responses are after 15-min intravenous infusions of isosmolar Ringer's buffer (*open circles*), hyperosmolar sucrose (*filled circles*), or hyperosmolar urea (*cross-hatched circles*) given at indicated times before airway instillation. Lung water was determined 2 h after acid instillation in each group. Mean ± SD. Each group comprises four experiments, except the 0.5-h group in which *open* and *filled circles* comprise five experiments each. *$p < 0.05$ compared with saline-instilled group.

Isolated Lung

K_{fc}. To determine K_{fc}, we adjusted vascular pressures to hold the isolated, blood-perfused lung under constant weight conditions. This was achieved by establishing pulmonary artery and left atrial pressures at approximately 7 and 3 cm H_2O, respectively. After baseline K_{fc} measurements, we gave a 15-min infusion of either isosmolar buffer or hyperosmolar sucrose in the perfusate. Then, 15 min after the end of the infusion, we instilled acid in the lung. After a subsequent 10 min, we determined K_{fc}.

For both the isosmolar and hyperosmolar groups, baseline K_{fc} was similar (Figure 6), and agreed with reported data (27). In the isosmolar group, acid instillation increased K_{fc} more than threefold (Figure 6), signifying marked increase in lung microvascular permeability. However, in the hyperosmolar group, no significant increase of K_{fc} occurred after acid (Figure 6), indicating that hyperosmolar pretreatment caused major inhibition of acid-induced microvascular injury.

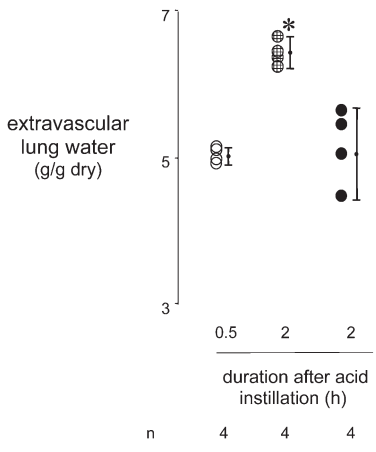

Figure 3. Postacid infusion of hyperosmolar sucrose. All groups received airway acid instillation. Thirty minutes after acid instillation, rats received either no infusion (*open circles*) or a 15-min intravenous infusion of either buffer (*cross-hatched circles*) or hyperosmolar sucrose (*filled circles*). At the indicated times after acid instillation, lungs were removed for lung water determination. Mean ± SD. n = number of experiments. *$p < 0.05$ compared with first group.

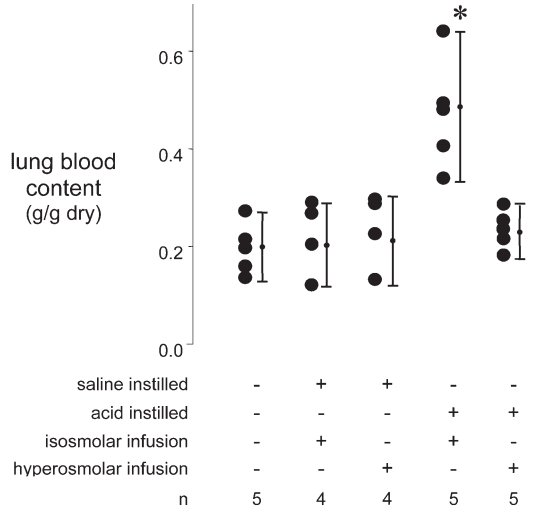

Figure 4. Effect of intravenous infusions on lung blood content. Group data are for baseline (*first group* of data points from *left*) and after a 15-min infusion of isosmolar Ringer's buffer or hyperosmolar sucrose, as indicated. Note that acid instillation increased lung blood content in the group that received isosmolar Ringer's buffer, but not in the group that received hyperosmolar sucrose. Mean ± SD. n = number of experiments. *$p < 0.05$ compared with baseline.

EVLW. To determine the effect of protecting leukocytes from hyperosmolar exposure, we perfused lungs with leukocyte-free blood for 15 min. Then, we infused either hyperosmolar sucrose or isosmolar buffer into the perfusion for 15 min. In the hyperosmolar group, at the end of the sucrose infusion, we replaced the sucrose-containing perfusate with isosmolar perfusate. After a further 15 min of perfusion with isosmolar perfusate, we added back leukocytes to the perfusate before instilling either acid or saline in the airway. Finally, we perfused the lung for 1 h further before assessing EVLW.

Figure 7 shows that as compared with control lungs that received no treatment (*first group* of data points), acid instillation markedly increased EVLW (*second group*). However, addition of hyperosmolar sucrose during leukocyte-free lung perfusion completely inhibited the EVLW increase (*third group*).

DISCUSSION

Our findings indicate that a brief intravenous infusion of hyperosmolar sucrose given before airway acid instillation effectively protected against acid-induced ALI. This was evident in that although intratracheal acid instillation induced ALI in the control group, as indicated in the increase of EVLW, a 15-min infusion of hyperosmolar sucrose given up to 1 h before or 0.5 h after acid instillation completely inhibited the EVLW increase. Furthermore, other indices of acid-induced ALI—namely, decrease of arterial Po_2 and increase of K_{fc}—were both markedly abrogated by pretreatment with hyperosmolar sucrose. Previously, we reported that the protection against acid-induced ALI occurs as a result of concurrent treatment with hyperosmolar sucrose (13). Our present findings indicate that the protection is achieved even when the hyperosmolar treatment is given before, or even after, acid instillation.

The protective effect of hyperosmolar sucrose was not due to exposure of leukocytes to hyperosmolarity. Thus, in the isolated, perfused lung, hyperosmolar infusion given under leukocyte-free conditions protected against acid-induced ALI. Because we removed leukocytes at the time of the hyperosmolar infusion, leukocytes were not exposed to hyperosmolarity; hence, hyperosmolarity-induced leukocyte effects (28, 29) were not present. Because leukocytes are required to establish ALI (2, 30), we added back leukocytes to the perfusing blood immediately before instilling acid. However, we believe that the hyperosmolar protective effect was already in place, possibly because of the direct barrier-enhancing effect of hyperosmolar sucrose that we previously reported in single lung microvessels (13). Here we show further evidence of this barrier-enhancing effect in that hyperosmolar sucrose effectively abrogated the acid-induced increase in K_{fc}. We suggest that hyperosmolarity-induced vascular barrier strengthening constituted the primary protective mechanism against acid-induced ALI.

The hyperosmolar protective effect was particularly well de-

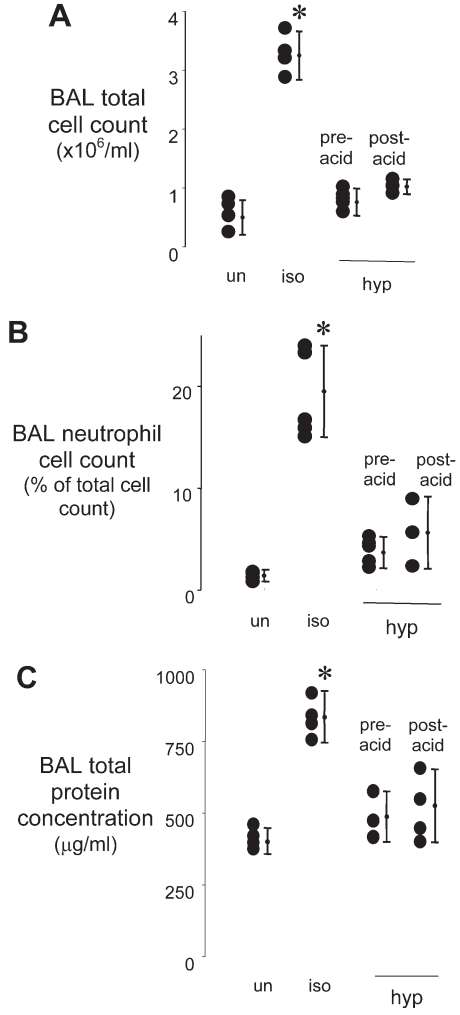

Figure 5. Effects of intravenous hyperosmolar therapy on bronchoalveolar lavage (BAL) cell count, neutrophil count, and protein concentration. Quantifications in the BAL are shown for groups given either no treatment or intravenous infusions of isosmolar Ringer's buffer or hyperosmolar sucrose. Each infusion was given for a 15-min period that ended 0.5 h before (*preacid)* airway acid instillation. Hyperosmolar sucrose was also given 0.5 h after (*postacid)* acid instillation. BAL was collected 2 h after acid instillation. Acid instillation increased total cell counts (*A*), neutrophil counts (*B*), and protein concentration (*C*) only in the group that received isosmolar Ringer's infusion. Mean ± SD. Each group comprises four experiments. iso = isosmolar Ringer's buffer; hyp = hyperosmolar sucrose; un = no treatment. *$p < 0.05$ compared with "no treatment" group.

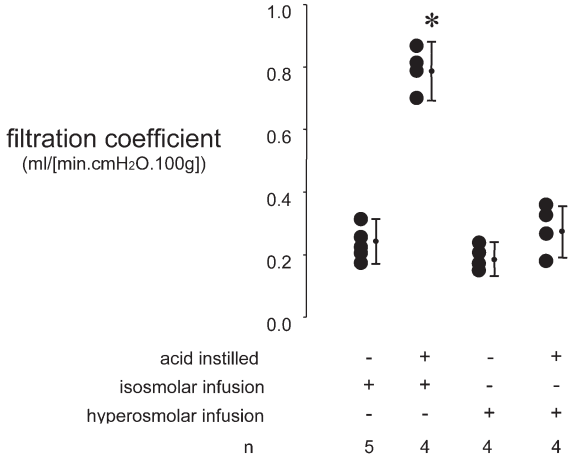

Figure 6. Filtration coefficient in isolated, perfused lungs. Group data show responses after either a 15-min isosmolar Ringer's or hyperosmolar sucrose infusion. Note that acid instillation increased filtration coefficient more than three times above control. Mean ± SD. n = number of experiments. *$p < 0.05$ compared with first group.

veloped for infusions given up to 1 h before acid instillation. Because we quantified EVLW 2 h after acid instillation, we estimate that the overall duration of the hyperosmolar protection was about 3 h. Accordingly, we were not able to achieve significant protection by giving the hyperosmolar infusion 2 h before acid instillation, probably because more than 3 h had elapsed. These findings are consistent with our previous findings that a 15-min exposure to hyperosmolar sucrose sustains antiinflammatory effects in endothelial cells and in single lung capillaries for at least 2 h (13, 14).

Major determinants of this sustained antiinflammatory effect are the increase in endothelial adherens junction assembly that

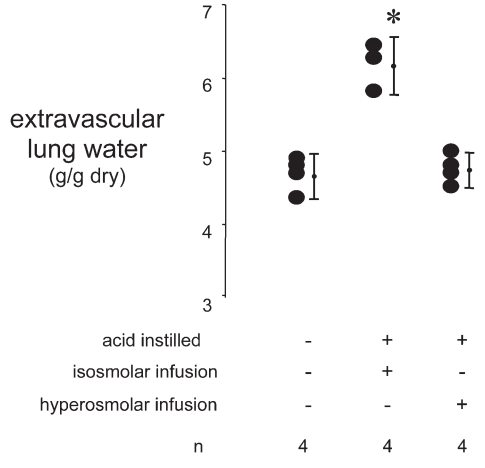

Figure 7. Extravascular lung water in isolated, blood-perfused lungs. The indicated infusions in different groups were given for 15 min while perfusing lungs with leukocyte-depleted blood. Leukocyte counts ($\times 10^3/mm^3$) in nondepleted and leukocyte-depleted blood were 4.5 ± 2.2 and 0.3 ± 0.2, respectively (n = 3). Perfusate osmolarities at before and at the end of the hyperosmolar sucrose infusion were respectively, 308 ± 14 and 366 ± 21 mOsm/kg (n = 3; $p < 0.05$). Leukocytes were added back to the perfusion 15 min before acid instillation. Mean ± SD. n = number of experiments. *$p < 0.05$ compared with the first group.

strengthens the endothelial barrier, and the increased endothelial cortical actin formation that inhibits expression of the leukocyte adherence receptor P-selectin (13, 14). Evidently, these protective responses occur as a result of hyperosmolar cell water extraction and are likely to depend on the cell impermeability of the osmolyte. Thus, because sucrose is cell impermeable, it was protective. However, because urea is cell permeable, it failed to be protective even at a hyperosmolar concentration. The somewhat longer duration of protection seen in the present experiments versus that in cultured cells (14) and single capillaries (13) suggests that the molecular barrier-enhancing mechanisms were better sustained in the composite lung vascular bed.

Although the pathophysiology of acid-induced ALI is not well understood, the contact of parenchymal tissue with concentrated acid is known to induce a progressive lung inflammation that causes time-dependent increases of EVLW. This was evident here in the small increase of EVLW 0.5 h after acid instillation, but a larger increase 2 h after. When we gave the hyperosmolar infusion 0.5 h after acid instillation, the increase in the 2-h postinstillation EVLW was almost completely blunted. We propose that after the initiation of ALI, early institution of hyperosmolar sucrose infusion prevented the ongoing lung injury that is characteristic of acid-induced ALI.

Indications of the inflammatory response induced by acid instillation were evident in the BAL data. Our BAL data are similar to previous reports (7, 25, 26), indicating that acid instillation increases hyperpermeability of the alveolocapillary barrier, thereby increasing both protein and inflammatory cell contents in the BAL. Importantly, both increases were completely inhibited by hyperosmolar infusions given either 0.5 h before or 0.5 h after acid instillation. These inhibitions affirm our view that a major effect of the hyperosmolar pretreatment was to strengthen the lung vascular barrier. We cannot rule out the possibility that similar priming effects also occurred in the alveolar wall.

A consistent finding in the protected groups in which hyperosmolar sucrose was given 0.5 or 1 h before acid instillation was that EVLW was lower than that in control lungs (Figure 2). Because the EVLW estimate is corrected for lung blood content, lung hemorrhage may cause an erroneous underestimate of EVLW. However, hyperosmolar sucrose did not alter lung blood content; hence, the lower EVLW was not the result of hyperosmolarity-induced lung hemorrhage. An explanation for this finding may be that plasma hyperosmolarity decreases the pericapillary interstitial pressure (13). Because the interstitial pressure varies directly with interstitial hydration (31), decrease of interstitial pressure denotes liquid removal from the pericapillary interstitium. We suggest that EVLW decreased to levels below control because of hyperosmolar water extraction from the extravascular space. Evidently, hyperosmolar pretreatment might protect against pulmonary edema by negatively regulating interstitial water.

In our report (13), the capillary hydraulic conductivity displayed a U-shaped relation to plasma osmolarity, predicting optimal barrier protection at vascular hyperosmolarity of 50 mOsm. Higher osmolarity reduced the barrier enhancement. Accordingly, here we targeted an increase of plasma osmolarity by approximately 50 mOsm by injecting concentrated sucrose in the femoral vein. The increase was transient, dissipating to baseline levels within 15 min, and did not cause any obvious deleterious effects such as local injury at the site of injection or any signs of respiratory distress. Importantly, these findings indicate that rapid dilution of the injected sucrose solution to the target levels of plasma hyperosmolarity is critical for achieving the protective effect. Excessive, sustained blood hyperosmolarity may itself be barrier damaging.

The present increase in lung blood content agrees with previous findings that red cell mass increases in acid-injured lungs

(24). Because we did not detect overt evidence for hemorrhage, such as blood-stained BAL, or hemorrhagic froth in the airway, the increased lung blood content may reflect a sustained increase of lung blood volume after acid injury. Although mechanisms remain incompletely understood, acid instillation might release arachidonate metabolites, such as thromboxane A_2, which cause lung venoconstriction (32, 33), thereby increasing capillary blood volume. Hence, as proposed previously (34), the present beneficial effects of hyperosmolar sucrose may accrue partially from relief of venoconstriction.

In conclusion, our findings indicate that slow infusion of a small volume of concentrated sucrose solution in the femoral vein induces safe, modest increases of plasma osmolarity, which protect against acid-induced lung inflammation. The protective effect was strongest when hyperosmolar sucrose was infused 1 h before the institution of acid injury. However, significant protection was also evident when the hyperosmolar infusion was given subsequent to acid instillation. Importantly, when given after acid instillation, the hyperosmolar infusion halted progressive injury. Whether the protective effect of hyperosmolar sucrose is extended over longer periods before and after induction of injury by repeating infusions remains unresolved. Nevertheless, our data indicate that hyperosmolar sucrose requires consideration as therapy in acid-induced ALI.

Conflict of Interest Statement: None of the authors have a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

References

1. Marik PE. Aspiration pneumonitis and aspiration pneumonia. *N Engl J Med* 2001;344:665–671.
2. Azoulay E, Attalah H, Yang K, Herigault S, Jouault H, Brun-Buisson C, Brochard L, Harf A, Schlemmer B, Delclaux C. Exacerbation with granulocyte colony-stimulating factor of prior acute lung injury during neutropenia recovery in rats. *Crit Care Med* 2003;31:157–165.
3. Goldman G, Welbourn R, Klausner JM, Kobzik L, Valeri CR, Shepro D, Hechtman HB. Leukocytes mediate acid aspiration-induced multiorgan edema. *Surgery* 1993;114:13–20.
4. Folkesson HG, Matthay MA, Hebert CA, Broaddus VC. Acid aspiration-induced lung injury in rabbits is mediated by interleukin-8-dependent mechanisms. *J Clin Invest* 1995;96:107–116.
5. Kawabata K, Hagio T, Matsumoto S, Nakao S, Orita S, Aze Y, Ohno H. Delayed neutrophil elastase inhibition prevents subsequent progression of acute lung injury induced by endotoxin inhalation in hamsters. *Am J Respir Crit Care Med* 2000;161:2013–2018.
6. Modelska K, Pittet JF, Folkesson HG, Courtney Broaddus V, Matthay MA. Acid-induced lung injury: protective effect of anti-interleukin-8 pretreatment on alveolar epithelial barrier function in rabbits. *Am J Respir Crit Care Med* 1999;160:1450–1456.
7. Nagase T, Ohga E, Sudo E, Katayama H, Uejima Y, Matsuse T, Fukuchi Y. Intercellular adhesion molecule-1 mediates acid aspiration-induced lung injury. *Am J Respir Crit Care Med* 1996;154:504–510.
8. Nagase T, Uozumi N, Ishii S, Kume K, Izumi T, Ouchi Y, Shimizu T. Acute lung injury by sepsis and acid aspiration: a key role for cytosolic phospholipase A2. *Nat Immunol* 2000;1:42–46.
9. Laffon M, Lu LN, Modelska K, Matthay MA, Pittet JF. Alpha-adrenergic blockade restores normal fluid transport capacity of alveolar epithelium after hemorrhagic shock. *Am J Physiol* 1999;277:L760–L768.
10. McAuley DF, Frank JA, Fang X, Matthay MA. Clinically relevant concentrations of beta2-adrenergic agonists stimulate maximal cyclic adenosine monophosphate-dependent airspace fluid clearance and decrease pulmonary edema in experimental acid-induced lung injury. *Crit Care Med* 2004;32:1470–1476.
11. Modelska K, Matthay MA, Brown LA, Deutch E, Lu LN, Pittet JF. Inhibition of beta-adrenergic-dependent alveolar epithelial clearance by oxidant mechanisms after hemorrhagic shock. *Am J Physiol* 1999; 276:L844–L857.
12. Nanjo S, Bhattacharya J, Staub NC. Concentrated albumin does not affect lung edema formation after acid instillation in the dog. *Am Rev Respir Dis* 1983;128:884–889.
13. Safdar Z, Wang P, Ichimura H, Issekutz AC, Quadri S, Bhattacharya J. Hyperosmolarity enhances the lung capillary barrier. *J Clin Invest* 2003;112:1541–1549.
14. Quadri SK, Bhattacharjee M, Parthasarathi K, Tanita T, Bhattacharya J. Endothelial barrier strengthening by activation of focal adhesion kinase. *J Biol Chem* 2003;278:13342–13349.
15. Parthasarathi K, Ichimura H, Quadri S, Issekutz A, Bhattacharya J. Mitochondrial reactive oxygen species regulate spatial profile of proinflammatory responses in lung venular capillaries. *J Immunol* 2002;169: 7078–7086.
16. Ichimura H, Parthasarathi K, Quadri S, Issekutz AC, Bhattacharya J. Mechano-oxidative coupling by mitochondria induces proinflammatory responses in lung venular capillaries. *J Clin Invest* 2003;111: 691–699.
17. Bhattacharya S, Sen N, Yiming MT, Patel R, Parthasarathi K, Quadri S, Issekutz AC, Bhattacharya J. High tidal volume ventilation induces proinflammatory signaling in rat lung endothelium. *Am J Respir Cell Mol Biol* 2003;28:218–224.
18. Bhattacharya J, Nakahara K, Staub NC. Effect of edema on pulmonary blood flow in the isolated perfused dog lung lobe. *J Appl Physiol* 1980; 48:444–449.
19. Selinger SL, Bland RD, Demling RH, Staub NC. Distribution volumes of [131I]albumin, [14C]sucrose, and 36Cl in sheep lung. *J Appl Physiol* 1975;39:773–779.
20. Bhattacharya J, Cruz T, Bhattacharya S, Bray BA. Hyaluronan affects extravascular water in lungs of unanesthetized rabbits. *J Appl Physiol* 1989;66:2595–2599.
21. Bhattacharya J, Gropper MA, Shepard JM. Lung expansion and the perialveolar interstitial pressure gradient. *J Appl Physiol* 1989;66: 2600–2605.
22. Effros RM. Osmotic extraction of hypotonic fluid from the lungs. *J Clin Invest* 1974;54:935–947.
23. Kanazawa M, Hasegawa N, Urano T, Sayama K, Tasaka S, Sakamaki F, Nakamura H, Waki Y, Terashima T, Fujishima S, *et al.* Regional lung hematocrit variation and assessment of acute lung injury. *J Appl Physiol* 1994;77:567–573.
24. Nader-Djalal N, Knight PR, Davidson BA, Johnson K. Hyperoxia exacerbates microvascular lung injury following acid aspiration. *Chest* 1997; 112:1607–1614.
25. Nishizawa H, Yamada H, Miyazaki H, Ohara M, Kaneko K, Yamakawa T, Wiener-Kronish J, Kudoh I. Soluble complement receptor type 1 inhibited the systemic organ injury caused by acid instillation into a lung. *Anesthesiology* 1996;85:1120–1128.
26. Van de Louw A, Jean D, Frisdal E, Cerf C, d'Ortho MP, Baker AH, Lafuma C, Duvaldestin P, Harf A, Delclaux C. Neutrophil proteinases in hydrochloric acid- and endotoxin-induced acute lung injury: evaluation of interstitial protease activity by in situ zymography. *Lab Invest* 2002;82:133–145.
27. Khimenko PL, Bagby GJ, Fuseler J, Taylor AE. Tumor necrosis factor-alpha in ischemia and reperfusion injury in rat lungs. *J Appl Physiol* 1998;85:2005–2011.
28. Rizoli SB, Rotstein OD, Parodo J, Phillips MJ, Kapus A. Hypertonic inhibition of exocytosis in neutrophils: central role for osmotic actin skeleton remodeling. *Am J Physiol Cell Physiol* 2000;279:C619–C633.
29. Rizoli SB, Kapus A, Parodo J, Rotstein OD. Hypertonicity prevents lipopolysaccharide-stimulated CD11b/CD18 expression in human neutrophils in vitro: role for p38 inhibition. *J Trauma* 1999;46:794–798. [Discussion: 798–789.]
30. Kyriakides C, Austen W Jr, Wang Y, Favuzza J, Moore FD Jr, Hechtman HB. Endothelial selectin blockade attenuates lung permeability of experimental acid aspiration. *Surgery* 2000;128:327–331.
31. Glucksberg MR, Bhattacharya J. Effect of dehydration on interstitial pressures in the isolated dog lung. *J Appl Physiol* 1989;67:839–845.
32. Wu W, Halebian PH, Hariri RJ, Cabrales SX, Shires GT, Barie PS. Differential effects of cyclo-oxygenase and thrombaxane synthase inhibition on ventilation-perfusion relationships in acid aspiration-induced acute lung injury. *J Trauma* 1992;33:561–567.
33. Shibamoto T, Wang HG, Yamaguchi Y, Hayashi T, Saeki Y, Tanaka S, Koyama S. Effects of thrombaxanxe A2 analogue on vascular resistance distribution and permeability in isolated-perfused dog lungs. *Lung* 1995;173:209–221.
34. Broe PJ, Toung TJ, Permutt S, Cameron JL. Aspiration pneumonia: treatment with pulmonary vasodilators. *Surgery* 1983;94:95–99.